ResearchGate

See discussions, stats, and author profiles for this publication at: https://www.researchgate.net/publication/303817350

Rapid ex situ culture of N-fixing soil lichens and biocrusts is enhanced by complementarity

Article *in* Plant and Soil · November 2016

DOI: 10.1007/s11104-016-2929-7

CITATIONS
24

READS
363

2 authors:

Matthew Alan Bowker
Northern Arizona University
165 PUBLICATIONS **12,192** CITATIONS

SEE PROFILE

Anita Antoninka
Northern Arizona University
45 PUBLICATIONS **1,019** CITATIONS

SEE PROFILE

Some of the authors of this publication are also working on these related projects:

Proyecto CYTED-EPES View project

Special Issue in Geoderma. Biocrusts: Engineers and Architects of Surface Soil Properties, Functions, and Processes in Dryland Ecosystems View project

All content following this page was uploaded by Anita Antoninka on 06 December 2017.

The user has requested enhancement of the downloaded file.

Plant Soil
DOI 10.1007/s11104-016-2929-7

REGULAR ARTICLE

Rapid ex situ culture of N-fixing soil lichens and biocrusts is enhanced by complementarity

Matthew A. Bowker · Anita J. Antoninka

Received: 15 January 2016 / Accepted: 18 May 2016
© Springer International Publishing Switzerland (outside the USA) 2016

Abstract
Background and aims Rehabilitation of biological soil crusts (biocrusts) in degraded drylands may facilitate ecosystem recovery. In order to rehabilitate biocrusts, ex situ culture methods for biocrust organisms must be optimized so that biocrusts may be grown in sufficient quantities to be reintroduced into degraded areas. Our goal was to improve these culture methods.
Methods We cultured six biocrust lichens and mosses, alone or in combinations, in a full-factorial greenhouse experiment, also manipulating water quality and hydration schedule.
Results All cultures produced a multi-species biocrust. The lichen *Collema* grew fastest, increasing by up to 238 % over 5 months. The mosses *Syntrichia caninervis*, and *Syntrichia ruralis* also grew, whereas other lichen species failed to maintain growth. Species combinations featuring *Collema* and both mosses exhibited greater growth rates for all species, compared to monocultures. All species were either unaffected by water quality, or performed better when irrigated with purer water. Several species responded favorably to shorter dry periods.
Conclusions The lichen *Collema* is a promising restoration material because of its culturability, and its N-fixation ability. Initial species composition of a culture will likely affect its success, and complementarity among species may be exploitable in order to produce inoculum faster.

Keywords Arid · Cryptobiotic soils · Ecological restoration · Nitrogen fixation · Semi-arid · Species interactions

Responsible Editor: Thom W. Kuyper .

Electronic supplementary material The online version of this article (doi:10.1007/s11104-016-2929-7) contains supplementary material, which is available to authorized users.

M. A. Bowker · A. J. Antoninka
School of Forestry, Northern Arizona University, 200 East Pine Knoll Drive, 86011, Flagstaff, AZ 86011, USA

M. A. Bowker (✉)
US Geological Survey, Southwest Biological Science Center, 2255 N. Gemini Drive, Flagstaff, AZ 86001, USA
e-mail: matthew.bowker@nau.edu

A. J. Antoninka
Department of Ecology and Evolutionary Biology, University of Colorado at Boulder, CB 334 Ramaley N285, Boulder, CO 80305, USA

Introduction

The drylands of the world present some of the more challenging land degradation problems, driving the expansion of the desert biome (Millennium Ecosystem Assessment 2005). It can be exceptionally difficult to rehabilitate degraded drylands for various reasons (Allen 1995), including: 1. The scarcity of resources–especially water– that recovering biota need (Bainbridge 2007), 2. Persistent dominance of invasive plants (Miller et al. 2011), and 3. The propensity for soil erosion to become a self-reinforcing process that precludes the recovery of desired biota (Ludwig and

Published online: 06 June 2016

Tongway 2000; Miller et al. 2012). Dryland rehabilitation approaches may have to focus not just on reintroducing desired biota, but also on restoring a system's ability to both capture and retain resources (Tongway and Ludwig 1996; Jacobs 2015) and resist invasion (Chambers et al. 2014). Drylands also are distinct in that there can be substantial interspaces between the canopies of grasses and shrubs, even when they are not degraded (Kéfi et al. 2007). The soil surface of these interspaces is the habitat of a highly functional assemblage of soil-aggregating organisms (cyanobacteria, mosses, lichens, fungi), which together compose biological soil crusts (biocrusts). Rehabilitation of biocrusts could benefit degraded drylands by increasing erosion resistance (Chaudhary et al. 2009), by creating and maintaining soil fertility (Reynolds et al. 2001; Belnap 2002), influencing the capture or redistribution of water (Eldridge et al. 2010; Chamizo et al. 2012), and discouraging some problematic exotic invasive plants (Serpe et al. 2006; Peterson 2013). A real or perceived weakness of biocrusts in a rehabilitation context is their variable but often slow natural recovery from disturbance (Weber et al. 2016), but recent research indicates that assisted recovery of biocrusts can be achieved (Wang et al. 2009; Liu et al. 2013; Lan et al. 2014). Development of viable and economical biocrust rehabilitation techniques can be viewed as an opportunity and a pathway to enhance the function of degraded drylands (Bowker 2007).

Biocrust rehabilitation is a young field that has only become a common research topic in the last ~15 years. A recent proliferation of research on this topic has emerged, especially from China (Tian et al. 2006; Li et al. 2010; Liu et al. 2013), but many questions remain unresolved (Zhao et al. 2016). Biocrusts can be rehabilitated in dryland ecosystems using salvaged natural biocrust material as inoculum (St. Clair et al. 1984; Belnap 1993). This practice may result in enhancement of ecosystem functions such as soil aggregation, and the creation of soil fertility (Maestre et al. 2006; Chiquoine et al. 2016). However, it is uncommon to have a ready supply of salvageable biocrust materials on hand. To fully realize the potential of biocrusts as a rehabilitation tool, ex-situ culture techniques must be developed and optimized (Bowker 2007; Zhao et al. 2016). With a few exceptions (Xu et al. 2008; Xiao et al. 2011), most work in this area has focused on culture of biocrust cyanobacteria with notable successes (Liu et al. 2013). Cyanobacteria may be isolated and grown in liquid media, which can be scaled up to produce inoculum in large quantities (Rao et al. 2009; Liu et al. 2013). Research on the other common biocrust autotrophs, mosses and lichens, has not progressed as far as the research on cyanobacteria (Zhao et al. 2016). Despite this, mosses are also generally culturable, either vegetatively from fragments of gametophytes or from spores, and can be grown on sand or agar substrates (Stark et al. 2004; Xu et al. 2008; Bu et al. 2011). Despite this knowledge, there remains much work to do to optimize the moss culture process to make it efficient enough to produce sufficient quantities of material for use in field applications. To our knowledge, rapid culture of dryland soil lichens has never been achieved and was therefore a much more notable research gap. Because some lichens are nitrogen fixers (Belnap 2002), it would be especially beneficial to overcome the technological hurdle of lichen culture, so that they may be artificially grown as a rehabilitation material (Bowker et al. 2010).

We sought to build on the emerging knowledge surrounding the production of biocrust inoculum in a greenhouse. Our experiments simultaneously answer questions about the culturability of various moss and lichen species, together and alone, while asking practical questions about optimal culture conditions in a greenhouse setting. Specifically, we tested the hypotheses that biocrust mosses and lichens grow best in combination with other community members, due to complementarity in growth requirements (Hector 1998). We also used our system to answer theoretical and practical questions about optimal culturing strategies for rapid culture of these putative slow growing organisms. Keeping the length of weekly hydration periods constant, we tested whether it was beneficial for growth to shorten dry periods in between hydration. Physiological experiments with desiccation tolerant desert mosses have identified length of desiccation period as one dimension of the stresses imposed by drying without dying (Proctor et al. 2007). We also tested the relative efficacy of de-ionized (DI) water and chlorinated tap water of generating biocrust growth, hypothesizing that chlorine would slow growth. Chlorinated tap water is common and inexpensive compared to DI water. If it is not detrimental to growth, there is value in using it. If these culture techniques are effective and refined, we may have a means to culture restoration-relevant amounts of biocrust materials necessary to rehabilitate lost ecosystem function in degraded drylands. Such a

technique could be a useful tool to combat land degradation, trigger favorable ecosystem state transitions, and reduce problematic dust emissions.

Materials and methods

Greenhouse-based culture system

We cultured biocrusts organisms on sand in an open system in a greenhouse as described in detail in Doherty et al. (2015). Briefly, our system employs a watering method based on capillary action. Timer-controlled pumps transport water to a lower plastic basin which drains slowly. Nested within the lower basin is another basin of identical size filled with a bed of sterile fine sand (80 ml), and several wicking holes in the bottom of the basin to allow entry of water via capillary action. Sand is used because it wicks water rapidly, and has been used successfully in the literature on moss culture (Xu et al. 2008), and our own previous research (Doherty et al. 2015). The sand (93.9 % sand, 5.5 % silt, 0.6 % clay) originated from a dune near Moab, Utah, and is derived from local sandstones originally. The pH of the soil is 8.4, and it is slightly effervescent. During a hydration event, a temporary water film is gently created at the soil surface, fully hydrating biocrust organisms. When the water flow terminates, both upper and lower basins drain to field capacity. From an experimental perspective, this system is ideal because an identical hydration status is produced at each watering event (field capacity), regardless of the pressure in water lines or location of the basins. This allows us the flexibility of designing well-replicated experiments with over 200 units, and true randomization of location of different treatments on greenhouse benches. Air temperature was maintained at 25 ± 3°. Relative humidity was not controlled and varied widely from 20 to 80 %. Light was also not controlled, and was about 60 % of outside PPFD, and varied over the 5 month experiment; all of the manipulated organisms have been observed in full sun and in partial shade in the field by the authors.

Experimental design

We established a full factorial experiment in this system in September 2014. In each experimental unit, we added field collected tissue of selected biocrust organisms. Our organism collections were sourced from a clay loam soil at Hill Air Force Base, Utah in the Great Basin of the USA. Species were removed from the soil surface in the field with knives and trowels, intentionally limiting the amount of underlying attached soil collected. We selected the following lichen species based on their prevalence at the site, and in the Great Basin and Colorado Plateau ecoregions: *Collema* spp. (mostly *C. tenax* (Sw.) Ach., but distinction from *C. coccophorum* Tuck. at this site is difficult), *Placidium* spp. (*Placidium squamulosum* (Ach.) Bruess, *P. lachneum* (Ach.) B. de Lesd., and *P. lacinulatum* (Ach.) Bruess often co-occur and field distinction is difficult), *Psora cerebriformis* W.A. Weber, *Psora decipiens* (Hedwig) Hoffm., and *Aspicilia hispida* Mereschk. All species have been observed by the authors to be able to grow on sandy substrates, indicating their compatibility with our culture system. In addition, we collected two moss species, *Syntrichia caninervis* Mitt. and *Syntrichia ruralis* Hedwig F. Weber & D. Mohr, both of which we had successfully grown in the greenhouse in the past (Doherty et al. 2015; Antoninka et al. 2016). These tissues were stored in an air dry state in the laboratory. To prepare them for use in our experiments we first gently removed as much soil as possible by hand and then crushed the specimens over a 2 mm mesh sieve, allowing soil to pass though while leaving the tissue of the organisms. The remaining materials were repeatedly wet-sieved over a 2 mm mesh sieve to remove the large majority of associated soil. The materials were air dried, then added by volume (10 ml total of material added) to the surface of each unit by sprinkling in a dispersed manner from a small measuring spoon. Each unit started at approximately 20 % cover of added biocrust material.

We manipulated 3 factors in a crossed design. The first factor was species composition, with the levels: *Syntrichia caninervis* alone, *Syntrichia ruralis* alone, *Collema* alone, *Placidium* alone, *Psora decipiens* alone, *Psora cerebriformis* alone, *Syntrichia caninervis* + *Syntrichia ruralis*, *Syntrichia caninervis* + *Syntrichia ruralis* + *Collema*, and uninoculated controls. Species combinations were added such that each species contributed an equal fraction of the total 10 ml added.

The second factor was watering schedule, with the levels: 3 days of continuous hydration, followed by a 4 day desiccation period (3/4), and 3 days of continuous hydration, followed by a 2 day desiccation period (3/2). A 3 day hydration period was chosen because a previous experiment comparing 2/5, 3/4, 4/3, and 5/2 schedules

found 3/4 most favorable for biocrust mosses (Antoninka et al. 2016). Varying the length of the desiccation period alters both the total accrued hydration time, which would be expected to benefit growth, and the number of potentially stressful drying events experienced. Thus, given a 3 day weekly hydration period, we chose to compare a schedule with the maximal desiccation period (3/4), to one with the minimum desiccation length to ensure complete air-drying of the soil (3/2).

The third factor was water quality, with the levels: deionized water, and municipal tap water (sourced seasonally from either reservoirs or wells, and chlorinated in a treatment plant). The city of Flagstaff, Arizona, reports the following properties of its municipal tap water, attributable to chlorination or toxic contaminants in 2014: Cl_2 (disinfectant) = 0.11–1.43 ppm, ClO_2 (disinfectant) = not detected - 350 ppb, haloacetic acids (disinfection byproduct) = not detected (<10) – 33 ppb, total trihalomethanes (disinfection byproduct) = not detected (<20) – 34 ppb, chlorite (disinfection byproduct) = not detected (<10 ppb), As =1.4–3.5 ppb, Ba^{2+} = 0.011–0.19 ppm, and Cr = not detected (City of Flagstaff 2014). Another city report lists additional properties of its various water sources: pH = 7.3–8.0, alkalinity =28.7–325 ppm, NO_3^- = <0.001 ppm, Ca^{2+} = 8.3 - 53 ppm, Mg^{2+} = 3.9 – 35 ppm, Na^+ = 3.6–10.5 ppm (City of Flagstaff 2016). Each treatment combination of the above factors was replicated 6 times, with the exception that uninoculated controls were replicated 3 times. This resulted in a total of 204 experimental units.

All treatments recieved 8 mL of Knop's solution (a dilute solution of macronutrients and some micronutrients; Reski and Abel 1985) monthly, following a watering event. Mosses respond favorably to the addition of macronutrients, such as N. This medium was the most effective of several in Xu et al. (2008) for culturing biocrust moss species, and the monthly application rate was the most effective in our previous work on biocrust mosses (Antoninka et al. 2016).

In addition to the main crossed experiment, we wished to learn more about some of the other species and possible species combinations, so we conducted a parallel pilot experiment in which we grew *Aspicilia hispida* alone, *Syntrichia caninervis* + *Syntrichia ruralis* + *Collema* + *Placidium*, and *Syntrichia caninervis* + *Syntrichia ruralis* + *Collema* + *Placidium* + *Aspicilia hispida.* We were limited by material in this case and grew each of these species compositions, unreplicated, in each combination of watering schedule and water type (totalling 12 experimental units). Both experiments were terminated after 5 months of growth.

Monitoring

We monitored percentage coverage monthly, using a circular gridded quadrat to aid in cover estimation. Mosses and lichens were identified to species level where possible. Non-target organisms, mostly cyanobacteria, also grew alongside the mosses and lichens. Cyanobacterial colonies were identified to genera based on morphology of the dominant species, omitting *Microcoleus* which was essentially always present but obscured because it grows underneath other taxa. Green algal colonies were generally not distinguished taxonomically, except for *Volvox* which had a distinct colony form.

Statistical analysis

We analyzed our monitoring data using repeated measures MANOVA. Total biocrust cover was analyzed in this way, based on the full experimental design. To learn more about individual species in the experiment, we selected contrasts of only those treatments in which a focal species was added, and subjected them to repeated measures MANOVA. This approach allowed us to learn species-specific increase rates and the degree to which they depended upon watering schedule or type, and on other species present.

We applied the approach of Loreau and Hector (2001) to numerically estimate the degree to which yield of target species in multi-species compositions deviated from that expected based on growth of constituent species in monoculture. This deviation from expected yield (ΔY) can be partitioned into a complementarity component, brought about by either species interactions or niche differentiation, or a sampling (a.k.a. selection) component, brought about by increasingly rich communities having a higher probability of containing a dominant species which will enhance or depress yield of the community.

Because a major part of biocrust growth was the expansion of non-target, but desirable cyanobacteria, we also conducted a multivariate community analysis. Target organisms were excluded from this analysis. September and October data were also excluded because cover was sparse, and samples lacking any cover of non-target organisms were frequent; in contrast, all

samples had some degree of non-target colonization in November–January. First we calculated the coverage of each taxon as a percentage of the area not occupied by target organisms. Second, we reduced the matrix using nonmetric multidimensional scaling (NMDS), selecting a 3 dimensional solution based on a Monte Carlo test (McCune and Grace 2002). The ordination was rotated so that the correlation of the dominant genus, *Nostoc*, was maximized with axis 1 ($r = 0.76$). This rotation also had the effect of creating a strong correlation of the genus *Scytonema* with axis 2 ($r = 0.88$), and of green algae with axis 3 ($r = .73$). Axis scores for each sample at each time point were saved and used as response variables in a structural equation model (SEM), as a function of the 3 main effects in our experiments. SEM is most often used as a causal inference tool because it allows researchers to test complex hypotheses regarding the hypothesized influences of multiple variables on one another (Shipley 2000; Grace 2006). It allows for a goodness of fit test which estimates the probability that a given dataset could plausibly arise from the causal hypothesis proposed by the researcher. Our goal was different. We instead emulated an ANOVA-type analysis so we could simultaneously estimate the relative effect sizes of the different experimental treatments on the 3 NMDS axes, across the later 3 time points. Effect size is measurable with the path coefficient, related and analogous to a partial correlation coefficient (Grace 2006). An R^2 can also be estimated for each response variable, reflecting the proportion of variance explained by factors in the model. Categorical predictors can be used in an SEM, simply if they are binary (e.g. water schedule and quality) but when they have multiple levels, such as composition, a composite variable is invoked (Grace 2006). Each of the 8 levels of composition (*Syntrichia ruralis* alone, *Syntrichia caninervis* alone, *Syntrichia caninervis* + *Syntrichia ruralis*, etc.) is coded as a binary dummy variable. The effects of each of these dummy variables are pooled by a composite to estimate the full effect of the factor composition. As a strategy to include an analysis of temporal changes, we used a multi-group procedure (Grace 2003), with the 3 months as groups. This procedure starts with the constraint that each path coefficient in the model is the same across all groups (e.g., the effect of water quality on axis 1 is the same in all 3 months). Usually, this is false, and the modeler relaxes these constraints one by one until a satisfactory model fit is obtained (Grace 2003). When a constraint is relaxed, a parameter, such as a path coefficient in one group, can be freely estimated separately from the other groups. Constrained parameters are instead estimated from the pooled data across all groups. In our case, the initial model with all paths constrained to be equal across months fit very well and was accepted as the final model.

Results

Overall biocrust cover

Overall, biocrusts gained cover over the course of the experiment (Fig. 1), and total cover was a mixture of added species and cyanobacteria and algae that were incidentally added in adherent soil or on the surface of lichens and mosses (see community analysis below). The percentage increase in cover increased monotonically through time ($F = 353.0$, $P < 0.0001$) from ~40 % in month 1 to ~225 % in month 5. Month 4 exhibited the fastest growth rate. Of all experimental factors, water quality was most influential as deionized water resulted in a greater increase in cover than tap water ($F = 61.1$, $P < 0.0001$); this difference was more pronounced in the final 2 months than the first 3 months ($F = 9.6$, $P < 0.0001$; Fig. 1a.). Species composition of inoculum had a strong effect on the eventual total cover ($F = 28.4$, $P < 0.0001$; Fig. 1b). The following generalities were observed: 1. Regardless of whether the target lichen actually grew, inoculation with lichens alone tended to result in greater total biocrust cover than inoculation with mosses alone. 2. In particular, inoculation of the lichen *Collema*, alone or in combination with mosses produced the greatest biocrust cover. The 3/2 watering schedule produced consistently greater growth, than the 3/4 schedule across all time periods ($F = 7.8$, $P = 0.006$; Fig. 1c.). There were additional weaker interactive effects between time and the experimental treatments, highlighted in Online Resource 1. Because our biocrusts were cultivated in an open system, there is opportunity for contamination by cyanobacteria and algae that did not originate from the biocrust inoculum. To estimate the magnitude of this effect, we also examined the total biocrust cover in uninoculated units through time. Although colonization did occur it was not until month 4 that control units attained a similar cover that inoculated units had at time 0 (Online Resource 1). Units inoculated with biocrusts generally supported several times the total biocrust cover as control units. Thus,

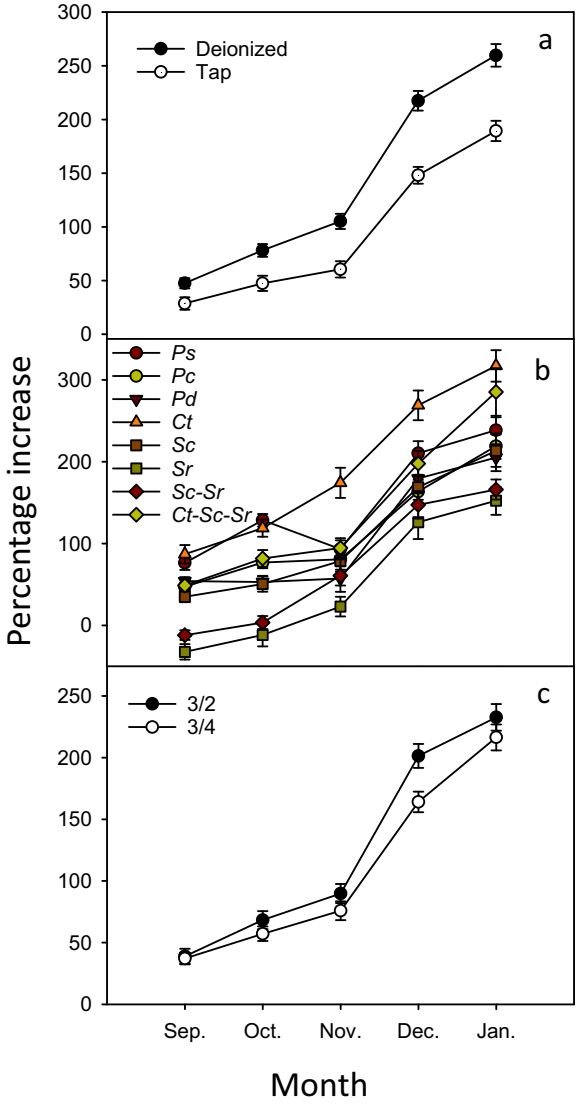

Fig. 1 Percentage increase in total biocrust cover through time from a starting point of 20 % cover (Time $F = 353.0$, $P < 0.0001$). **a** Effect of water quality on percentage increase of biocrust cover (Water Quality $F = 61.1$, $P < 0.0001$; Water Quality × Time $F = 9.6$, $P < 0.0001$). **b** Effect of species composition of starter inoculum on percentage increase of biocrust cover (Composition $F = 28.4$, $P < 0.0001$). **c** Effect of irrigation schedule on percentage increase of biocrust cover (Schedule $F = 7.82$, $P = 0.006$). Species codes: Ps = *Placidium*, Pc = *Psora cerebriformis*, Pd = *Psora decipiens*, Ct = *Collema*, Sc = *Syntrichia caninervis*, Sr = *Syntrichia ruralis*. Irrigation schedules: 3/2 = Three day hydration periods alternating with 2 day desiccation periods, 3/4 = Three day hydration periods alternating with 4 day desiccation periods

although contamination is occurring, it appears highly unlikely to be driving major treatment differences observed above.

Performance of individual target species

Three species, *Syntrichia caninervis*, *Syntrichia ruralis* and *Collema* all increased cover over the course of the experiment, and maintained positive growth (Fig. 2). *Collema* cover was by far the most responsive over time. Treatment effects on these three species are discussed below. *Placidium*, *Psora decipiens*, and *Psora cerebriformis* all initially exhibited net growth for the first 1–2 months of the experiment, but declined sharply afterward and experienced a net loss in cover (Fig. 2). Water quality and desiccation length had no effects on these species.

Planned contrasts allowed us to track the response of the three best performing species to our treatments through time. Below we highlight the strongest effects on growth of individual species. A full accounting of all model effects is presented in Online Resource 2.

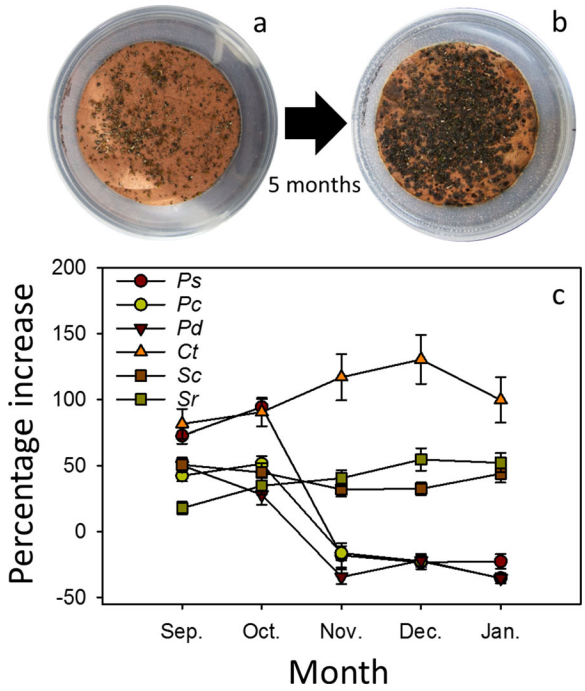

Fig. 2 Percentage increase over time of target species from a starting point of 20 %. **a** An experimental unit, inoculated with *Collema* at time zero. **b** The same experimental unit, after 5 months. Cover is dominated by *Collema* along with cyanobacteria. **c** Growth of target species over time (expressed as % change in cover). *Collema* was the best performer, while *Syntrichia* spp. both maintained positive growth, and other lichens failed to maintain positive growth. Ps = *Placidium*, Pc = *Psora cerebriformis*, Pd = *Psora decipiens*, Ct = *Collema*, Sc = *Syntrichia caninervis*, Sr = *Syntrichia ruralis*

Syntrichia caninervis increased across all treatments by 151 % in the best performing treatment. The strongest effect on its rate of growth was species composition ($F = 22.8$, $P < 0.0001$; Fig. 4a). Growth rate was lower on average when *Syntrichia caninervis* was growing in combination with *Syntrichia ruralis* than when growing alone. In contrast, growth was about ~1.3 × higher in the three species combination (*Syntrichia caninervis* + *Syntrichia ruralis* + *Collema*), than when *Syntrichia caninervis* was growing alone. Water quality was also an important factor ($F = 19.2$, $P < 0.0001$), with higher growths rates observed when watered with deionized as opposed to tap water (Fig. 3a. These treatments diverged gradually through time ($F = 9.6$, $P < 0.0001$), such that at month 1, growth rates were similar, but by month 5 treatments irrigated with deionized water had nearly 3× the increase in cover as those irrigated with tap water. The 3/2 treatment resulted in a slower rate of growth in *Syntrichia caninervis* than the 3/4 treatment, which was the opposite of the effect observed for total biocrust cover.

Syntrichia ruralis increased by over 100 % in several treatments. This species was most strongly influenced by water quality (F = 78.27, $P < 0.0001$; Fig. 3b); growth rates were ~1.3× faster across all time periods when deionized water was used rather than tap water. Species composition was also highly influential as *Syntrichia ruralis* attained similar growth rates growing alone or in combination with *Syntrichia caninvervis* but grew ~150 % faster across all time periods when growing in combination with both *Syntrichia caninervis* and *Collema* (Fig. 4b). These two factors also interacted ($F = 5.37$, $P = 0.007$) such that the growth rate of *Syntrichia ruralis* when watered with deionized water and growing in the three species composition was always substantially higher than any other combination of these factors. The growth rate also varied through time ($F = 4.61$ $P = 0.003$), increasing in months 4 and 5 after being nearly static from months 1–3.

Collema increased by up to 238 % in several treatments. The interaction between time and composition was the most influential factor ($F = 7.24$, $P = 0.0004$). In most time points *Collema* grew at a faster rate of increase when growing in combinations with *Syntrichia* mosses, except November (Fig. 4c). Time was also influential ($F = 5.73$, $P = 0.001$), with most of the increase apparently occurring in between time 0 and 1 month, with a more gradual general increase in cover in months 2–5. Finally, a main effect of water quality

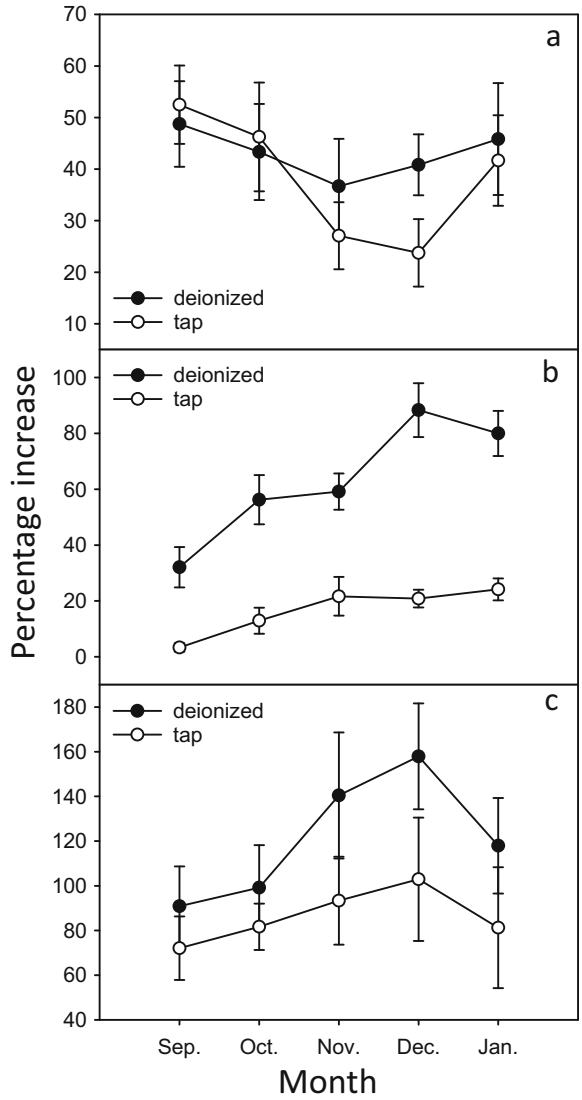

Fig. 3 Percentage increase in total cover of the three best-performing target species through time as a function of water quality. Percentage increase of *Syntrichia caninervis* cover (Water Quality $F = 19.2$, $P < 0.0001$). **b** Percentage increase of *Syntrichia ruralis* cover (Water Quality $F = 78.3$, $P = <0.0001$). **c** Percentage increase of *Collema* cover (Water Quality $F = 4.3$, $P = 0.04$)

was also detected (F = 4.3, $P = 0.04$; Fig. 3c), whereby *Collema* grew approximately 25 % faster when irrigated with deionized water rather than tap water.

Complementarity and sampling effects

Our multispecies compositions differed in deviation from expected yield. *Syntrichia caninervis* + *Syntrichia ruralis* + *Collema* treatments tended to overyield more

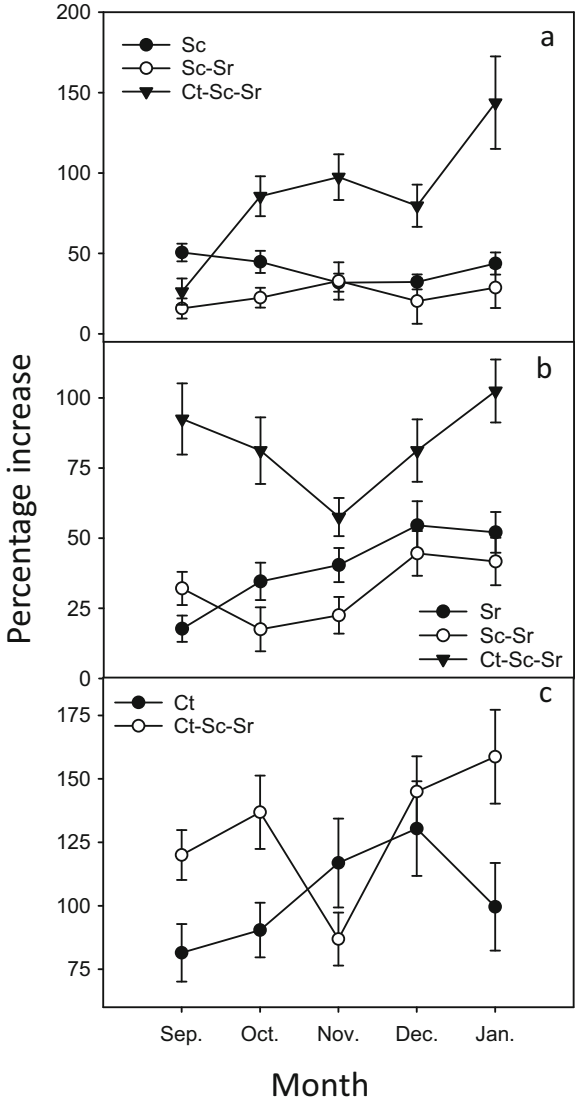

Fig. 4 Percentage increase in total cover of the three best-performing target species through time as a function of starting composition of inoculum. **a** Percentage increase of *Syntrichia caninervis* cover (Composition $F = 22.8$, $P < 0.0001$). **b** Percentage increase of *Syntrichia ruralis* cover (Composition $F = 5.4$, $P = 0.007$). **c** Percentage increase of *Collema* cover (Time × Composition $F = 7.2$, $P = 0.0004$). Species codes: Ct = *Collema*, Sc = *Syntrichia caninervis*, Sr = *Syntrichia ruralis*

than the *Syntrichia caninervis* + *Syntrichia ruralis* treatments (Table 1), though ΔY depended on water quality and schedule. More striking was that positive complementarity effects largely accounted for observed ΔY in most *Syntrichia caninervis* + *Syntrichia ruralis* + *Collema* treatments, whereas positive sampling effects accounted for ΔY in most *Syntrichia caninervis* + *Syntrichia ruralis* treatments (Table 1).

Non-target community analysis

Model fit of our initial model was satisfactory ($X^2 = 193.109$, $P = 0.44$; note in this goodness of fit test, the probability of model fit is estimated, therefore higher rather than lower *P*-values are desired), thus we did not relax any constraints. This indicates no temporal interactions between experimental factors and NMDS axes, and therefore an identical model was estimated which applied to all 3 months. The experimental factors explained 21 % of the variation in axis 1, 24 % in axis 2, and 44 % in axis 3 (Fig. 5). Axis one (correlated with *Nostoc*) was influenced by all three factors at a small to medium magnitude. Higher water quality increased axis 1 scores ($r = 0.16$), and the 3/4 schedule decreased scores compared to the 3/2 schedule ($r = -0.31$). The effect of species composition ($r = |0.29|$; note that composite paths do not have an interpretable sign, and are presented as absolute values) was largely attributable to a negative effect of *Psora decipiens* inoculum on *Nostoc* abundance, and a positive effect of *Collema* inoculum on *Nostoc*. The strongest influences on axis 2 (correlated with *Scytonema*) were due to species composition ($r = |0.38|$), most strongly attributable to a positive effect of *Placidium* inoculum on *Scytonema*. Effects of water quality and schedule on axis 2 were similar to those on axis 1. Axis three (correlated with green algae) was distinct from the other axis in that is was strongly affected by water quality ($r = -0.55$), such that tap water addition promoted green algae. Species composition was also influential ($r = |0.37|$), and was driven by an inhibitory effect of inocula containing *Collema* on green algae. Finally, the 3/4 schedule weakly suppressed green algae ($r = -0.11$).

Discussion

Rapid culture of a biocrust lichen

We successfully cultured the lichen *Collema* in a greenhouse setting. *Collema* and the two *Syntrichia* species stand out in our experience as the most culturable species to date in our greenhouse system (Table 2). We are not aware of any other example of the artificial rapid increase of a dryland soil lichen. There is a practice of "gardening" using both mosses and lichens for landscaping purposes, but to our knowledge, these are techniques that are more applicable to mesic environments

Table 1 Deviation from expected yield (ΔY), complementarity and sampling effects in our multi-species compositions (Loreau and Hector 2001). Sc = *Syntrichia caninervis*, Sr = *Syntrichia ruralis*, Ct = *Collema*. Complementarity and sampling effect sum to ΔY

Composition	Water quality	Water schedule	ΔY	Complementarity	Sampling effect
Sc - Sr - Ct	De-ionized	3/2	24.1	29.4	−5.4
	De-ionized	3/4	19.3	19.8	−0.5
	Tap	3/2	10.9	10.9	−0.1
	Tap	3/4	1.7	2.7	−1.1
Sc - Sr	De-ionized	3/2	−3.1	−3.1	0.1
	De-ionized	3/4	14.9	−5.8	20.7
	Tap	3/2	11.5	−0.3	11.8
	Tap	3/4	9.9	−4.7	14.6

(Schenk 1997). There also exists a literature on the culture of lichen bionts, but the fungal biont is slow-growing in culture. For example grams of mycobiont tissue can be cultured in 8–16 month periods (Honegger et al. 1992). Lichens have been resynthesized by the combination of separately cultured bionts (Ahmadjian 1959), but this technique is more applicable to basic research questions than rapid culture.

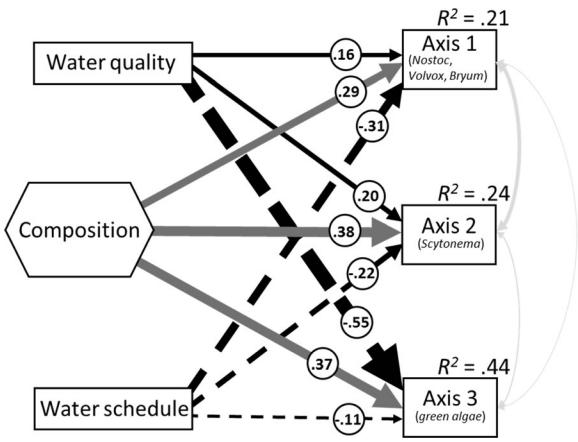

Fig. 5 Structural equation model depicting relative influence of experimental treatments on non-target species. Rectangles indicate directly measured variables, and the hexagon represents a composite variable. Axes 1–3 are NMDS axis scores for each sample summarizing variation in the non-target community. Axis 1 correlates primarily with *Nostoc* (r = 0.76), *Volvox* (r = .69), *Bryum* sp. (r = .43). Axis 2 correlates primarily with *Scytonema* (r = .88). Axis 3 correlates primarily with green algae (r = .73). *Arrows* represent an influence of one variable upon another. *Dashed black arrows* represent negative effects and *solid black arrows* represent positive effects. Effects of the composite variable "composition" have no interpretable sign, thus corresponding *arrows* appear in *dark gray*. Double-headed, light *gray arrows* represent residual correlations between the 3 axes. Arrow widths are scaled proportionally to the strength of the estimated relationship which is measured by the path coefficient, appearing in *circles*

Most of the lichens tested did not grow well under greenhouse conditions, with the sole exception being *Collema*. *Collema* has great potential as a restoration target species because it is a nitrogen fixer, and is early successional among biocrust lichens (Belnap 2002; Bowker et al. 2010). Past work has focused on various methods to assist its growth in the field, or determine the identity of its limiting factors so that they may be augmented in the field (Davidson et al. 2002; Bowker et al. 2005, 2006, 2008, 2010). One experiment attempted resynthesis of *Collema* lichens in the field via the addition of spores and cultured photobionts (Davidson et al. 2002). New thalli were in fact promoted by spore addition, but this finding does not lend itself easily to restoration applications because there is no practical method to produce spores. All previous research has taken the path of attempting to promote *Collema* growth or colonization in the field, rather than producing *Collema* tissue vegetatively in the greenhouse for later field application.

Not only did *Collema* grow, but it attained substantially higher growth rates than any other target organism, making it the most efficient starting material for biocrust inoculum that we have investigated in our greenhouse system (Fig. 2). Furthermore, it appears to facilitate the growth of other species, meaning that a mixed culture containing *Collema* will grow at a faster rate than a single species culture. This result is exemplified by the fact that both moss species examined, *Syntrichia ruralis* and *Syntrichia caninervis* grew fastest when in a 3 species combination along with *Collema* (Fig. 3c). Finally, cultures containing *Collema*, both alone or with other species, attain the greatest total cover of biocrust, and promote desired N-fixers such as *Nostoc*, while suppressing likely greenhouse-sourced invaders such as green algae (Fig. 4).

Table 2 Amenability to culture of 5 biocrust lichens and 2 biocrust mosses, expressed as maximal percentage increase in cover. Data are treatment means for the present study unless: [a] unreplicated pilot experiment, [b] from Antoninka et al. 2016. No fertilizer was added unless: [c] 4 fertilization treatments were tested, water quality was not tested

Spicies	Maximum rate of increase Conditions	Notes
Lichens		
Aspicilia hispida	160 % 2 months, Ah, De-ionized, 3/2	Growth strongly declines after initial months
Collema	238 % 5 months, Sc-Sr-Ct, De-ionized, 3/2	
Collema	620%[a] 4 months, Sc-Sr-Ct-Ps, De-ionized, 3/4	
Placidium	111 % 2 months, Ps, De-ionized, 3/2	Growth strongly declines after initial months
Psora Cerebriformis	60 % 2 months, Pc, Tap, 3/2	Growth strongly declines after initial months
Psora Cerebriformis	80%[a] 3 months, Sc-Sr-Ct-Pc-Pd-Ps, Tap,3/2	
Psora Decipiens	63 % 1 month, Pd, Tap, 3/2	Growth strongly declines after initial months
Mosses		
Syntrichia ruralis	165 % 1 month, Sc-Sr-Ct, De-ionized, 3/2	Growth not sustained
Syntrichia ruralis	320%[a] 5 months, Sc-Sr-Ct-Cs-Pd-Pc, De-ionized, 3/4	
Syntrichia ruralis	450%[b] 5 months, 2/4, biweekly fertilizer[c]	
Syntrichia caninervis	248 % 5 months, Sc-Sr-Ct, De-ionized, 3/4	
Syntrichia caninervis	420%[a] 5 months, Sc-Sr-Ct-Cs, De-ionized, 3/4	
Syntrichia caninervis	475%[b] 5 months, 2/4, biweekly fertilizer[c]	

We can only speculate as to why *Collema* grew and other lichens failed to grow. *Collema* may simply be better than other lichens at vegetative reproduction, related to its earlier successional arrival, or it may be more tolerant of the greenhouse environment (substrate, light, microclimate), related to its wider distribution. The other lichens may have performed poorly because they cannot maintain high photosynthetic rates at warm temperatures. For example, *Psora cerebriformis* attains its photosynthetic optimum between about 10–28 °C, while *Collema tenax* does so between 26 and 36 °C. While thermostat control would have prevented air temperatures from exceeding 28, soil surface temperatures were likely warmer, favoring *Collema* over *Psora* spp. or *Placidium*. Additional trials manipulating temperature and other factors may be informative, and could lead to successful culture of these species (Lange et al. 1997, 1998).

Practical considerations of hydration in biocrust greenhouse culture

In all cases, higher water quality either had no effect or exerted a positive effect on growth rates compared to tap water. On one hand, tap water contains nutrient ions which may benefit biocrusts, as we found in prior experiments that manipulated fertilizer application (Antoninka et al. 2016). On the other, municipal tap water of the United States is generally treated with chlorine, which could plausibly build up to toxic concentrations, and in our region may contain enough carbonates that availability of some immobile nutrients is reduced (Lajtha and Schlesinger 1988). A preferred approach clearly is the use of the more pure water source, and addition of nutrients separately as desired. We cannot determine from our data if deionized water is necessary, or simply superior to chlorinated tap water. There are additional, potentially faster and cheaper ways to purify water such as charcoal filters which remain untested. Furthermore, other regions have tap water that is not chlorinated or which is not alkaline, and this type of water also remains untested.

Watering schedule influenced the growth rate and eventual composition of biocrusts in our culture system. We have imposed at least one desiccation period each week in this and prior experiments (Doherty et al. 2015; Antoninka et al. 2016). Our rationale was that this practice would be likely to reduce external contamination because organisms originating from sources other than biocrusts would be less likely to be desiccation tolerant. Given this constraint, an array of manipulations of hydration schedule are possible. The length of both hydration period, and desiccation period might be

important parameters (Coe et al. 2012). Longer hydration periods provide opportunities for greater activity and positive carbon gain (Coe et al. 2012). The ability of desiccation tolerant organisms to fully recover activity after a desiccation decreases the longer they have been desiccated (Proctor et al. 2007). In an earlier experiment, focused on the mosses *Syntrichia ruralis* and *Syntrichia caninervis*, we focused on simultaneously increasing hydration period length and decreasing desiccation period length (Antoninka et al. 2016). We found, after comparing 2/5, 3/4, 4/3, and 5/2 weekly wet/dry schedules that moss growth was optimized by 3/4, but incidental cyanobacteria were favored by longer hydration periods. The current experiment instead held the weekly hydration period length constant, but varied the length of the desiccation period from 2 to 4 days. Although less influential than water quality, the schedule was an important factor for some species. *Syntrichia caninervis* growth was better in a 3/4 schedule, while total biocrust cover (mostly cyanobacteria) increased faster under a 3/2 schedule. We hypothesize that this result is related to the relative ability of biocrust organisms to survive desiccation events, especially under warm temperatures typical of a greenhouse. While the 3/2 schedule results in greater cumulative activity time over the course of 5 months, it also accrues a larger number of desiccation events that even for desiccation tolerators may be stressful. *Syntrichia caninervis* is known to be sensitive to repeated drying events under warm conditions, and may even experience mortality (Reed et al. 2012). In contrast, if cyanobacteria such as *Nostoc* are relatively more resistant to this stressor, they might benefit due to the longer cumulative activity time of the 3/2 schedule. Clearly, it is difficult to separate the effects of total hydration time, and the number of desiccation events, since they tend to correlate in most logical experimental designs. Further experiments varying these factors independently will be needed to more fully understand the interplay between these different elements of the hydration schedule.

A role for diversity and species interactions in biocrust greenhouse culture

As mentioned previously, different species compositions of inocula differentially influence the total amount of biocrust grown in a fixed period of time. Often, species richness is asymptotically, positively related to productivity of a community (Tilman et al. 1996). This pattern may arise in experiments due to 2 mechanisms: 1. Complementarity of functional traits of species (Hector 1998), 2. A sampling effect wherein the more species are added to a community, the greater the chances are of it including a highly productive species (Wardle 1999). Using total biocrust cover as a measure of community productivity, it seems clear that overall productivity was greatest when *Collema* was present, regardless if it was added alone or with mosses. This result appears consistent with the sampling effect mechanism (Wardle 1999). However, a closer look at the increase of particular species suggests a more interesting set of dynamics based on complementarity and species interactions.

If the increase of particular species is a goal, there appear to be facilitative interactions to be harnessed, and competitive interactions to be avoided. This point is well illustrated by *Collema*, and both *Syntrichia* species. When all three species were co-inoculated in equal amounts, the growth rate of all three species was enhanced compared to each species growing alone. Complementarity was by far the strongest positive determinant of deviation from expected yield in the three species treatments, while sampling effects were relatively small and negative (Table 1). Perhaps the nitrogen-fixing ability of *Collema* or the N-fixing cyanobacteria it promoted boosted the nitrogen availability for the mosses, and perhaps the water retention of the mosses slowed water loss rates in *Collema* during desiccation events. Such complementarity of functional traits might lead to species coexistence or facilitation. We also grew both *Syntrichia* species together and observed a neutral to negative effect of these two species on one another. In this case, complementarity was a weak negative determinant of deviation for expected yield in the *Syntrichia caninervis* + *Syntrichia ruralis* communities, indicating some competitive inhibition among species (Table 1). The sampling effect, instead was a stronger positive driver of deviation from expected yield. The *two Syntrichias*, as con-generics, might be somewhat redundant rather than complementary, which could set the stage for a competitive interaction.

Although statistical inference is impossible due to lack of replication, our pilot experiment offers some additional insight regarding possible complementary, facilitative or inhibitory effects. When this triad of species is growing with a fourth, *Placidium*, growth of *Collema* and both *Syntrichias* is even greater than in

the 3 species combination. The addition of 2 more species, the lichens *Psora cerebriformis* and *Psora decipiens* in a 6 species combination also results in strong growth of both mosses, but decreases *Collema* growth to less than that observed in the 3 species combination. All of these examples serve to illustrate the following generalizations: 1. Growth of a target organism may be enhanced or suppressed when growing with another organism, 2. Even if one organism fails to grow in culture, its addition may influence the growth of other organisms, 3. A more complete understanding of the interactions among a set of species will lead to superior culture practices. Our goal here was not to exhaustively explore the effect of starter inoculum composition, but this factor proved to be quite important among our treatments. Exhaustive trials of all possible species compositions in a replicated design may be an area in which we can most efficiently optimize the practice of producing mixed biocrust cultures. These results also highlight the utility of our experimental system in conducting basic research using biocrusts as a model system (Bowker et al. 2014).

A final hypothetical outcome of diversity in cultured biocrusts is greater probability of success in field application. The end product of our culturing activities is a mixed species biocrust, enriched in mid-late successional species such as target *Collema* and *Syntrichia* spp., but also containing substantial biomass of early successional cyanobacteria. Regardless of the successional state of the ecosystem or the particular stressors there, introduction of more species enhances the probability that at least one of them will be successful. Field survival of different inocula will provide a test of this hypothesis, and may lead to a valuable new restoration material which can restore function to degraded drylands.

Acknowledgments We gratefully acknowledge funding from the Strategic Environmental Research and Development Program (Grant number RC- 2329; Department of Defense, Department of Energy, and Environmental Protection Agency), Northern Arizona University's Faculty Grants Program, and the Bureau of Land Management Colorado State Office. Technical assistance was provided by Jeff Wright, Hilda Smith, and Phil Patterson. We thank Hill Air Force Base Utah Test and Training Range, and Russell Lawrence (Natural Resources Division) for facilitating access to field sites. We thank Drs. Thomas Kuyper, Ted Melis, Barbara Ralston, Jim Grace, and two anonymous reviewers for constructive feedback on early drafts. Use of trade, product, or firm names is for information purposes only and does not constitute an endorsement by the U.S. Government.

References

Ahmadjian V (1959) A contribution toward lichen synthesis. Mycologia 51:56–60

Allen EB (1995) Restoration ecology: limits and possibilities in arid and semiarid lands. In: Roundy BA, MacArthur ED, Haley JS, and Mann DK (Comps.) Proceedings: Wildland shrub and arid lands restoration symposium. General Technical Report INT-GTR-315. US Department of Agriculture, Forest Service, Intermountain Research Station, Ogden, Utah, pp. 7–15

Antoninka AJ, Bowker MA, Reed SC, Doherty KD (2016) Production of greenhouse-grown biocrust mosses and associated cyanobacteria to rehabilitate dryland soil function. Rest Ecol 24:324–335

Bainbridge DA (2007) A guide for desert and dryland restoration. Island Press, Washington, DC

Belnap J (1993) Recovery rates of cryptobiotic crusts: Inoculant use and assessment methods. Great Basin Nat 53:89–95

Belnap J (2002) Nitrogen fixation in biological soil crusts from southeast Utah, USA. Biol Fert Soils 35:128–135

Bowker MA (2007) Biological soil crust rehabilitation in theory and practice: an underexploited opportunity. Rest Ecol 15: 13–23

Bowker MA, Belnap J, Davidson DW, Phillips SL (2005) Evidence for micronutrient limitation of biological soil crusts: potential to impact aridlands restoration. Ecol Appl 15:1941–1951

Bowker MA, Belnap J, Davidson DW, Goldstein H (2006) Correlates of biological soil crust distribution across a continuum of spatial scales: support for a hierarchical conceptual model. J Appl Ecol 43:152–163

Bowker MA, Koch GW, Belnap J, Johnson NC (2008) Nutrient availability affects pigment production but not growth in lichens of biological soil crusts. Soil Biol Biochem 40: 2819–2826

Bowker MA, Belnap J, Davidson DW (2010) Microclimate and propagule availability are equally important for rehabilitation of dryland N-fixing lichens. Rest Ecol 18:30–33

Bowker MA, Maestre FT, Eldridge DJ, Belnap J, Castillo-Monroy AP, Escolar C, Soliveres S (2014) Biological soil crusts (biocrusts) as a model system in community, landscape, and ecosystem ecology. Biodivers Conserv 23:1619–1637

Bu CF, Yang JZ, Zhang XC (2011) Cultivation experiment of moss plants from biological soil crusts in Mu Us sandy land. Chinese J Des Res 31:937–941

Chambers JC, Bradley BA, Brown CS, D'Antonio C, Germino MJ, Grace JB, Hardegree SP, Miller RF, Pyke DA (2014) Resilience to stress and disturbance, and resistance to *Bromus tectorum* L. invasion in cold desert shrublands of western North America. Ecosystems 17:360–375

Chamizo S, Cantón Y, Lázaro R, Solé-Benet A, Domingo F (2012) Crust composition and disturbance drive infiltration through biological soil crusts in semiarid ecosystems. Ecosystems 15: 148–161

Chaudhary VB, Bowker MA, O'Dell TE, Grace JB, Redman AE, Johnson NC, Rillig M (2009) Untangling the biological controls on soil stability in semi-arid shrublands. Ecol Appl 40:2309–2316

Chiquoine, LP, Abella, SR and Bowker, MA (2016) Rapidly restoring biological soil crusts and ecosystem functions in highly disturbed desert ecosystems. Ecol Appl doi: 10.1002/15-0973

City of Flagstaff (2014). City of Flagstaff consumer confidence reports: Public water system (PWS) information. <http://www.flagstaff.az.gov/DocumentCenter/View/46103>

City of Flagstaff (2016) Water quality data and distribution. Unpublished letter available to water customers

Coe KK, Belnap J, Sparks JP (2012) Precipitation-driven carbon balance controls survivorship of desert biocrust mosses. Ecology 93:1626–1636

Davidson DW, Bowker MA, George D, Phillips SL, Belnap J (2002) Treatment effects on performance of N-fixing lichens in disturbed soil crusts on the Colorado plateau. Ecol Appl 12:1391–1405

Doherty KD, Antoninka AJ, Bowker MA, Velasco Ayuso S, Johnson NC (2015) A novel approach to cultivate biocrusts for restoration and experimentation. Ecol Rest 33:13–16

Eldridge DJ, Bowker MA, Maestre FT, Alonso P, Mau RL, Papadopoulos J, Escudero A (2010) Interactive effects of three ecosystem engineers on infiltration in a semi-arid Mediterranean grassland. Ecosystems 13:499–510

Grace JB (2003) Comparing groups using structural eqs. In: Pugesek BH, Tomer A, and von Eye A (eds.), Structural equation modeling: applications in ecology and evolutionary biology. Cambridge University Press, Cambridge, U.K., pp. 281–296.

Grace JB (2006) Structural equation modeling and natural systems. Cambridge University Press, Cambridge, U.K., 378 pp

Hector A (1998) The effects of diversity on productivity: detecting the role of species complementarity. Oikos 82:597–599

Honegger R, Kutasi V, Ruffner HP (1992) Polyol patterns in eleven species of aposymbiotically cultured lichen mycobionts. Mycol Res 97:35–39

Jacobs BF (2015) Restoration of degraded transitional (piñon-juniper) woodland sites improves ecohydrological condition and primes understory resilience to subsequent disturbance. Ecohydrology 8:1417–1428

Kéfi S, Alados CL, Pueyo Y, Papanastasis VP, ElAich A, de Ruiter PC (2007) Spatial vegetation patterns and imminent desertification in Mediterranean arid ecosystems. Nature 449:213–217

Lajtha K, Schlesinger WH (1988) The effect of $CaCO_3$ on the uptake of phosphorous by two desert shrub species, *Larrea tridentata* (DC.) Cov. and *Parthenium incanum* H.B.K. Bot Gaz 149:328–334

Lan S, Zhang Q, Wu L, Liu Y, Zhang D, Hu C (2014) Artificially accelerating the reversal of desertification: cyanobacterial inoculation facilitates succession of vegetation. Environ Sci Technol 48:307–315

Lange OL, Belnap J, Reichenberger H, Meyer A (1997) Photosynthesis of green algal soil crust lichens from arid lands in southern Utah, USA: role of water content on light and temperature responses of CO_2 exchange. Flora 192:1–15

Lange OL, Belnap J, Reichenberger H (1998) Photosynthesis of the cyanobacterial soil crust lichen *Collema tenax* from arid lands in southern Utah, USA: role of water content on light and temperature responses of CO_2 exchange. Funct Ecol 12: 195–202

Li XR, Tian F, Jia RL, Zhang ZS, Liu LC (2010) Do biological soil crusts determine vegetation changes in sandy deserts? Implications for managing artificial vegetation. Hydrol Process 24:3621–3630

Liu YD, Chen LZ, Hu CX (2013) Environmental biology of desert cyanobacteria and combating desertification with biological soil crust. Science Press, Beijing

Loreau M, Hector A (2001) Partitioning selection and complementarity in biodiversity experiments. Nature 412:72–76

Ludwig JA, Tongway DJ (2000) Viewing rangelands as landscape systems. In: Arnalds O, Archer S (eds) Rangeland desertification. Kluwer Academic Publishers, Dordrecht, pp. 39–52

Maestre FT, Martın N, Díez B, López-Poma R, Santos F, Luque I, Cortina J (2006) Watering, fertilization, and slurry inoculation promote recovery of biological crust function in degraded soils. Microbial Ecol 52:365–377

McCune B, Grace JB (2002) Analysis of ecological communities. MJM Software Design, Gleneden Beach, Oregon

Millennium Ecosystem Assessment (2005) Ecosystems and human well-being: desertification synthesis. World Resources Institute, Washington, DC

Miller ME, Belote RT, Bowker MA and Garman SL (2011) Alternative states of a semiarid grassland ecosystem: Implications for erosion susceptibility, ecosystem services and management. Ecosphere 2:Art 55

Miller ME, Bowker MA, Reynolds R, Goldstein H (2012) Post-fire land treatments and wind erosion – lessons from the Milford flat fire. Aeolian Res 7:29–44

Peterson EB (2013) Regional-scale relationships among biological soil crusts, invasive annual grasses, and disturbance. Ecol Process 2:2

Proctor MCF, Wood AJ, Alpert P, Stark LR, Cleavitt NL, Mishler BD (2007) Desiccation tolerance in bryophytes: a review. Bryologist 110:595–621

Rao BQ, Liu YD, Hu CX, Li DH, Shen YW, Wang WB (2009) The technology of man-made algal crust and its applications in control of desertification. Acta Hydrobiol Sin 33:756–761

Reed SC, Coe KK, Sparks JP, Housman DC, Zelikova TJ, Belnap J (2012) Changes to dryland rainfall result in rapid moss mortality and altered soil fertility. Nat Clim Chang 2: 752–755

Reski R, Abel WO (1985) Induction of budding on chloronemata and caulonemata of the moss, *Physcomitrella patens*, using isopentenyladenine. Planta 165:354–358

Reynolds R, Belnap J, Reheis M, Lamothe P, Luiszer F (2001) Aeolian dust in Colorado plateau soils: nutrient inputs and recent change in source. Proc Nat Acad Sci 98:7123–7127

Schenk G (1997) Moss gardening: including lichens, liverworts, and other miniatures. Timber Press, Inc., Portland, Oregon

Serpe MD, Orm JM, Barkes T, Rosentreter R (2006) Germination and seed water status of four grasses on moss-dominated biological soil crusts from arid lands. Plant Ecol 185: 163–178

Shipley B (2000) Cause and correlation in biology: a user's guide to path analysis, structural equations and causal inference. Cambridge University Press, Cambridge, UK

St. Clair LL, Webb BL, Johansen JR, Nebeker GT (1984) Cryptogamic soil crusts: enhancement of seedling establishment in disturbed and undisturbed areas. Reclam Reveg Res 3:129–136

Stark LR, Nichols L, McLetchie DN, Smith S, Zundel C (2004) Age and sex-specific rates of leaf regeneration in the Mojave Desert moss *Syntrichia caninervis*. Am J Bot 91:1–9

Tian GQ, Bai X, Xu J, Wang X (2006) Experimental studies on the natural restoration and the artificial culture of the moss crusts on fixed dunes in the Tengger Desert. China Front Biol 1:13–17

Tilman D, Wedin D, Knops J (1996) Productivity and sustainability influenced by biodiversity in grassland ecosystems. Nature 379:718–720

Tongway DJ, Ludwig JA (1996) Rehabilitation of semiarid landscapes in Australia. I. Restoring productive soil patches. Rest Ecol 4:388–397

Wang W, Liu Y, Li D, Hu C, Rao B (2009) Feasibility of cyanobacterial inoculation for biological soil crusts formation in desert area. Soil Biol Biochem 41:926–929

Wardle DA (1999) Is "sampling effect" a problem for experiments investigating biodiversity–ecosystem function relationships? Oikos 83:403–407

Weber B, Bowker MA, Zhang Y and Belnap J (2016) Natural recovery of biological soil crusts after disturbance. In: Weber, B, Büdel, B, and Belnap, J (eds.), Biological soil crusts: an organizing principle in drylands. Ecological Studies Series. Springer-Verlag, Berlin, pp 479–498

Xiao B, Wang Q, Zhao Y, Shao M (2011) Artificial culture of biological soil crusts and its effects on overland flow and infiltration under simulated rainfall. Appl Soil Ecol 48:11–17

Xu S, Yin C, He M, Wang Y (2008) A technology for rapid reconstruction of moss-dominated soil crusts. Env Engineering Sci 25:1129–1137

Zhao Y, Bowker MA, Zhang Y and Zaady E (2016). Enhanced recovery of biological soil crusts after disturbance. In: Weber, B, Büdel, B, Belnap, J (eds.), Biological soil crusts: an organizing principle in drylands. Ecological Studies Series. Springer-Verlag, Berlin, pp. 499–523

View publication stats